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Subject: Public Comments on Arkema Inc.'s HPV Challenge Program Test Plan for **Dimethyl Disulfide** (DMDS, CAS# 624-92-O)

The following comments on Arkema Inc.'s test plan for dimethyl disulfide are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than ten million Americans.

Dimethyl disulfide is used in the oil industry as a sulfiding/presulfiding agent to activate catalysts of hydrotreating units, to reduce the number of decoking operations in the petrochemical industry, as a chemical intermediate in the fine chemical industry, and as an anticorrosive in metallurgy.

We commend Arkema Inc. for its comprehensive review of existing data, which identified reliable data for dimethyl disulfide for all mammalian toxicity endpoints. Arkema fulfills the reproductive toxicity endpoint with a weight-of-evidence approach using data from the histopathology of reproductive organs from a 28-day repeated dose study in combination with a negative developmental study, thus avoiding a checklist approach to toxicology. This is a scientifically valid analysis that is accepted by the OECD and has been recommended by the EPA in a number of previous test plans.

Arkema Inc. is proposing to conduct an OECD 203 acute fish toxicity test, which will kill approximately 120 animals. We detail below several methods by which Arkema can either model this testing or reduce the number of fish used and we urge Arkema and the EPA to consider these alternative methods to satisfy the acute fish toxicity endpoint for dimethyl disulfide.

The EPA guidance document "The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program" notes that if a QSAR model is available, it may be used with the appropriate rationale for its applicability to the HPV candidate chemical and identifies ECOSAR as an available model to estimate aquatic ecotoxicity. We urge Arkema Inc. and the EPA to consider the applicability of ECOSAR to satisfy the acute fish toxicity endpoint for dimethyl disulflde.

In a recent publication, the Ecotoxicology Task Force of the European Center for the Validation of Alternative Methods (ECVAM) described a method with the potential to reduce the number of fish used in ecotoxicity testing for chemical substances by 53.6%-71.2%.[1] Noting that fish are less sensitive than algae or daphnia in acute aquatic toxicity tests roughly 85% of the time*, a fish acute threshold (step-down) method was developed. An upper threshold concentration (UTC) is set at the lowest $EC_{50}$ value observed in the algae and daphnia tests. An acute test is carried out at the UTC using five test and five control fish. If no toxicity is observed, no further tests are carried out and the acute fish toxicity result ($LC_{50}$) is reported as greater than the UTC value. If toxicity is observed, a second test is performed at a step-down concentration using a dilution factor of 3.2, based on a semi-logarithmic concentration series. The testing continues to lower concentrations until no toxicity is observed. The $LC_{50}$ 96-hour value can be obtained from all step-down threshold test data by applying the binominal method of interpolation. An additional refinement could be obtained by terminating the test after 24 hours of exposure, when lethality and/or serious morbidity are observed in two out of five fish. We strongly urge the use of this new testing strategy when no replacement for the acute fish toxicity test is perceived to be applicable.

In vitro test methods to replace the acute fish toxicity test are also available. The validated DarT Test[3] uses fertilized zebrafish eggs as a surrogate for living fish. Since the eggs do not hatch during the test period, the DarT is classified as a non-animal test. The exposure period is 48 hours, and assessed endpoints include coagulation, development of blastula, gastrulation, termination of gastrulation, development of somites, movements, extension of the tail, development of eyes, heartbeat, circulation, heart rate, pigmentation, and edema. Endpoints comparable to lethality in vivo include failure to complete gastrulation alter 12 hours, no 16 hours, no heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of the effects of test substances. The reliability and relevance of the DarT test have been confirmed through an international, multi-laboratory validation study coordinated and financed by the German Environmental Protection Agency. Predictions of acute toxicity from the DarT test were highly concordant with in vivo reference data? This in vitro test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent[5], and has since been nominated for development into an OECD test guideline. It is clearly suitable for immediate use as a replacement for the use of fish in SIDS screening studies.

Another promising in vitro assay is TETRATOX. In this assay, the protozoan Tetrahymena pyriformis is used as a biomarker for acute lethality in fish.[6] The biochemistry and physiology of T. pyriformis have been thoroughly investigated since the 1950s, and this assay has been used, in various forms, for aquatic toxicity testing since the 1970s.[7] In this test, a range-finding study followed by three replicate definitive tests is performed for each test substance. Each treatment replicate consists of a minimum of five different concentrations per substance

tested. Thus, at least 30 data 'points make up each analysis. The current, standardized protocol is for a 40 hour static test, which provides for multigenerational exposure. Range-finding tests are also included to allow an accurate approximation of both the highest concentration with no observed effect on population growth and the lowest concentration with total inhibition of cell replication. Output measures from the TETRATOX assay are the 50% inhibitory growth concentration (IGC50, mmol/L) and the 95% fiducial interval. The current TETRATOX database includes more than 2,000 industrial organic chemicals, including over 800 aliphatic chemicals, 900 aromatic chemicals, 400 neutral narcotics, and 400 direct-acting electrophiles, among others. The TETRATOX protocol has now been standardized and has undergone a preliminary ring test.' The German EPA is currently funding a second, more elaborate ring test, with the goal of establishing an OECD test guideline. In the meantime, data generated by TETRATOX demonstrate a consistently high degree of concordance with data from in vivo acute studies in fish, which supports the use of this assay as a replacement for toxicity studies in fish.[9]

Thank you for your attention to these comments. I may be reached at 610-586-3975, or via e-mail at josephm@peta.org.

Sincerely,

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Research & Investigations

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